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(54) Title: RECOMBINANT VACCINE

(57) Abstract

A method for an induction of immune response against polypeptide employing antigenic presentation of said polypeptide in the form of either fusion protein with MHC product amino acid sequence or tertiary complex with MHC product on natural or artificial membrane, where said membrane may further either localize in internal compartment mediators of immune response or present membrane-bound form of said mediators on the surface of said membrane.

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Description

Recombinant Vaccine

Technical Field

The present invention relates, generally, to genetically engineered vaccines and use of these vaccines for induction of the specific immunity. More particularly, the present invention relates to the recombinant fusion proteins useful for induction of the cell and/or humoral immune response as well as for immunotherapy.

Description of the Prior Art

It is generally known that induction of specific immune response against pathogen efficiently protects animals as well as humans from disease induced by this pathogen. Such pathogens may be viruses, bacteria, parasites and neoplasia.

The crucial role of the specific protective immunity is well established. Vaccination against the common infectious agents, as it was progressively implemented during the present century, has been very important, especially for the highly contagious

infections. Two major groups of vaccines include attenuated live preparations of the infectious agents and different forms of vaccinating antigens. A majority of these variants is reviewed (G. L. Ada, Vaccines in Fundamental Immunology, W.E. Paul, Raven Press, N.Y., 1989, pp. 985-1057). Recent advances in biomedical studies provided background for identification of the proteins and peptide potentially enable to induce protective immune response against various pathogens.

The major difference between attenuated live vaccines and various preparations of the potential vaccinating antigens has been understood recently. This difference relates to the different pathways of the antigen presentation during the antigen processing by host immune system. Live viral vaccines are immunologically processed through MHC class I restricted immune response inducing preferential and highly efficient T-cell mediated immunity. At the same time soluble antigen preparations are processed through MHC class II restricted response inducing almost exclusively humoral immunity with low protective efficiency. Unfortunately, the number of infection agents do not

allow the use of attenuated vaccines and various approaches were proposed to resolve the problem using inactivated pathogens, subunit vaccines based on natural proteins, recombinant proteins and synthetic peptide.

Recombinant proteins providing immune response against envelope viral proteins (U.S. Patent No. 4,790,987), Hepatitis B Virus protein (U.S. Patent No 5,019,386), Fowpox Virus protein (U.S. Patent No. 5,093,258), Herpes Simplex Virus protein (U.S. Patent No 4,859,587), Parainfluenza (U.S. Patent No. 4,847,081), Melanoma-specific protein (U.S. Patent No 5,141,742) and many others have been disclosed. Synthetic peptide as the source of antigenically active determinants inducing immune response against pathogen were disclosed for malaria (U.S. Patent No. 4,957,738), Hepatitis B Virus (U.S. Patent No. 4,778,784), Human Immunodeficiency Virus (U.S. Patent No 4,957,737; U.S. Patent No. 5,081,226).

In order to overcome the weak activity and low efficiency of the vaccine antigens, different approaches were used. These approaches include degradable microspheres as delivery systems (U.S. Patent No. 5,160,745), administration of the vaccine

proteins in the immobilized form (U.S. Patent No 5,045,320) or in the form of immobilized antigen-antibody immune complexes (U.S. Patent No. 4,493,825), copolymerization of antigenic peptide (U.S. Patent No. 4,957,738) and administration of the mixture of the potential vaccine with either adjuvant (U.S. Patent No 5,047,238; U.S. Patent No. 4,590,181) or cytokines (U.S. Patent No. 4,689,224). However, all these ways do not provide an efficient combination for protein or synthetic peptide vaccine. Even in vivo expression of the vaccine protein using recombinant vaccinia virus (U.S. Patent No. 5,077,213) has failed to increase the immunological activity of the potentially active protein.

The practical use of the vaccine antigen preparations is limited by their inadequate low efficiency in induction of the broad protective humoral and/or cell immune response as compared with live vaccines. It is clear that if the difficulties encountered in the development of the individual particular vaccines could be overcome on common basis, a major advancement in the treatment of various diseases, including AIDS, could be achieved.

The ideal vaccine preparation should provide

cell and humoral immune response supporting efficient protection against pathogen. Therefore, the method for vaccine development should be applied to various pathogens.

The present invention provide the method for construction of the recombinant vaccine proteins and their use for induction of the protective immune response.

Disclosure of Invention

It is, therefore, an object of the present invention to provide a common basis for development of the recombinant proteins which overcomes the disadvantages inherent in the prior art and are effective in the immunoprophylactic and immuno-therapeutic treatment of infection and other diseases through induction of pathogen-specific immunity.

It is an additional object of the present invention to provide a rational method for using of recombinant vaccine proteins for a treatment of infection and other diseases when recovery of said diseases is associated with induction of the specific immune response.

Other objects and advantages of the present

invention will become apparent as the description thereof proceeds.

The foregoing and related objects of the present invention are achieved by induction of the allo-specific-like cell and humoral immune response against antigenic target polypeptide sequence. Said presentation of the target sequence is achieved using either [1] direct incorporation of the target sequence in the sequence of the MHC product or [2] association of the target sequence with MHC product through noncovalent binding or [3] indirect association of the target sequence with MHC product in the cluster of the membrane proteins.

Recombinant proteins containing the target amino acid sequence are expressed in suitable prokaryotic or eukaryotic genetic system. Amino acid sequence homologous to the leader peptide of eukaryotic membrane protein should be included into the expressed sequence for direct expression of the recombinant protein on the membrane of eukaryotic cells using any recombinant expression vector. The foregoing recombinant vaccine protein is essentially presented to the host immune system in the membrane-bound form on the surface of either natural cell or

artificial membrane of the natural or artificial vesicles. Said vesicles may contain cytokines and mediators necessary for the local regulation of the immune reaction.

The foregoing vaccine protein, in accordance with the present invention, should essentially include either [1a] transmembrane amino acid sequence, [1b] amino acid sequence providing interaction with T-lymphocyte MHC product receptors and [1c] target amino acid sequence or [2a] anchor amino acid sequence, providing an association of said target amino acid sequence with MHC product and [2b] target amino acid sequence or [3a] amino acid sequence of cell membrane protein associated in membrane cluster with MHC product and [3b] target amino acid sequence or [4a] anchor structure providing association of the target sequence with cell protein in the membrane cluster with MHC product and [4b] target amino acid sequence.

[1a] Said transmembrane amino acid sequence may be either transmembrane domain of any membrane protein or amino acid sequence homologous to transmembrane region of any membrane protein.

[1b] Said amino acid sequence providing

interaction with MHC product receptor may be either CD8 binding sequence derived from the amino acid sequence of the MHC class I product or CD4 binding sequence derived from amino acid sequence of the MHC class II product.

[2a] Said anchor amino acid sequence may be, but is not limited to, the ß-2-microglobulin sequence providing interaction with MHC class I cell membrane protein, amino acid sequence of MHC class II alpha-chain or ß-chain sequences providing interaction correspondingly with ß-chain or alpha-chain MHC class II cell membrane proteins providing interaction with cell membrane proteins.

[3a] Said amino acid sequence may be complete or partial sequence of any membrane protein able to form a membrane cluster with MHC product including but not limited to receptors of growth factors, lymphokines and cytokines. Said sequence should essentially include the transmembrane region for membrane expression of recombinant protein.

[4a] Said anchor structure may be formed by any receptor-binding structure including, but not limited to, the receptor binding peptide and polypeptides of growth factors, lymphokines, cytokines and hormones.

In accordance with the present invention, the recombinant vaccine protein may be either used for immunization in association with the membrane of the eukaryotic expressing cells or purified. Purified recombinant vaccine protein should be essentially incorporated into the natural cell or artificial membrane. Foregoing recombinant vaccine protein should be expressed under the control of the corresponding genetic regulatory elements in the bacterial, yeast or eukaryotic cells including cells of the host subjected for immunization by said recombinant protein.

Foregoing recombinant protein should be essentially bound to natural or artificial membrane, incorporated into the natural or artificial membrane or expressed on the natural cell membrane. Said membrane may be artificial membrane of the liposomes, membrane of the erythrocytes and erythrocyte ghosts, membrane of nucleated cells or vesicles produced from any kind of membranes.

Recombinant vaccine protein produced by fusion of the target antigenic sequence with MHC class II sequence may be further presented on the membrane carrying membrane-bound form of Interleukin-1.

To overcome the inefficient immune response of immunocompromised host, liposomes and erythrocyte ghosts presenting recombinant vaccine protein may further contain cytokines and local mediators of immune response including but not limited to Interleukin 2, Interleukin 4 and Interleukin 5 in concentrations which are sufficient for the local induction of differentiation and proliferation of the immunocompetent cells. Further increase of the host immune response may be achieved through combination of cytokines and local immunoregulators with central regulators of immune system including but not limited to thymopoietins, thymosins and their active peptide fragments.

In accordance with the present invention, recombinant vaccine protein produced by the fusion of the antigenic target amino acid sequence with either anchor amino acid sequence or anchor structure should be essentially used as a membrane bound complex of the recombinant protein with cell membrane protein. Said complex may be produced either in vivo or in vitro. Formation of immunizing protein complex in vivo may be achieved using either in vitro expression of recombinant protein following by interaction in

vivo with corresponding host membrane protein or in vivo expression of recombinant vaccine protein in cells of immunized host by appropriate expression vectors. Said vectors may be, but are not limited to, vaccinia virus based vectors and retroviral vectors. Said vectors are used either for infection of immunized host or infection of the cells derived from the host following by immunization of the host by infected cells. Other noninfectious genetically engineered expressing vectors may be used for transfection of the host-derived cells and expression of the vaccine protein following by immunization of the host by either transfected cells or their membranes.

Formation of the immunizing complex in vitro may be achieved by interaction of the recombinant vaccine protein through an anchor amino acid sequence or anchor structure with corresponding cell membrane protein wherein said membrane protein should be essentially bound to natural or artificial membrane, incorporated into the natural or artificial membrane or expressed on the natural cell membrane. Said membrane may be an artificial membrane of the liposomes, membrane of the erythrocytes and

erythrocyte ghosts, membrane of nucleated cells or membrane vesicles produced from any kind of membranes. Artificial liposomes, membrane vesicles and erythrocyte ghosts presenting immunizing complex of recombinant vaccine protein with cell membrane protein may further contain cytokines, local mediators of immune response and central immuno-regulators including but not limited to Interleukin 2, Interleukin 4, Interleukin 5, thymopoietins, thymosins and their biologically active peptide fragments in concentrations which are sufficient for the local induction of differentiation, proliferation of the immunocompetent cells and immunocorrection of the host immune response.

The present invention should be understood as an artificial protein construction preferentially providing an induction of the immunological effector mechanisms through either direct or indirect association of the target antigenic amino acid sequence with MHC product amino acid sequence. For the purposes of achieving the objects of the present invention, recombinant protein constructed from MHC class I product with substitution of the hyper-variable region(s) of alpha-1 and/or alpha-2 domains

by target antigenic sequences, provides the most effective recombinant vaccine when folded in the complex with beta-2-microglobulin and expressed on the cells of an immunized host.

Examples of the recombinant proteins constructed in accordance with the present invention include, but are not limited to, Example I: fusion protein with substitution of the alpha helix in the alpha-1 domain of MHC class I protein by target amino acid sequence; Example II: fusion protein with substitution of the alpha-1 and alpha-2 domains of the MHC class I protein by target amino acid sequence; Example III: fusion protein of ß-2 microglobulin anchor sequence with target sequence in the complex with MHC class I protein; Example IV: fusion protein of the ß-2 microglobulin anchor sequence with target sequence in the form of membrane protein containing transmembrane domain; Example V: fusion protein with substitution of alpha-1 domain of the alpha-chain of MHC class II protein; Example VI: fusion protein with substitution of ß-1 domain of the ß-chain of MHC class II protein.

The following Example VII demonstrates the construction of the recombinant vaccine protein with target sequence of HIV-I env product gp41 using

substitution of the alpha-helix in the alpha-1 domain of the MHC class I product by amino acid sequence derived from gp41 according to Example I.

Example VII:

1. Peripheral blood mononuclear cells are separated from the donor blood by Ficoll-Paque density centrifugation. Cells are stimulated in culture by PHA for four days and total RNA was prepared using the guanidinium thiocyanate-phenol-chloroform extraction method [Chomczynsky P., and Sacchi N. Analytical Biochemistry 162:156-158 (1987)].

2. cDNA corresponding to the leader peptide of the MHC class I product is prepared from the total PBMC RNA using Avian Myeloblastosis Virus reverse transcriptase and the cDNA Kit (Promega) as described by the manufacturer in a total volume of 20 µl using 15 pM of the primer A AAT ACC tcT aGA GTG GGA GCC (positions from 73 to 95 relatively to the A of the ATG codon of human MHC-A,B,C, primer contains mutations to create XbaI restriction site). The reverse transcription reaction is carried out at +42°C for 1 hour. The enzyme is inactivated at +95°C for 15 min and the reaction mixture is adjusted to

the Taq polymerase reaction containing 0.05 M Tris buffer pH 8.3, 0.2 mM each of the four dNTP, 2.0 mM MgCl2, 50 mM KCl, 15 pM of the upstream primer TCGGATcCTCCCCAGACGCCGAGG ATG (positions from -24 to 3 relatively to the A of the ATG codon of human MHC-A, primer contains mutation to create BamHI restriction site) and 2.5 U of the AmpliTaq polymerase (Perkin-Elmer Cetus). The PCR reaction is subjected to 30 cycles of amplification using the following path: +95°C for 60 sec, +50°C for 60 sec, +72°C for 60 sec.

3. cDNA corresponding to the MHC class I coding sequence corresponding to alpha-2, alpha-3, trans-membrane and cytoplasmic domains is prepared as described in Step 1 and amplified as described in Step 2 using primers CCCACAGtCgaCTGTCTCA GGC TTT (1093-1119, SalI) and GC TAC TAC AtC tAG AGC GAG GCC GGG (320-345, XbaI) correspondingly.

4. PCR-amplified cDNA's are restricted by corresponding endonucleases and purified after electrophoresis in low melting agarose using Magic Prep PCR Kit from Promega according to instructions of Manufacturer. Purified fragments are cloned into the corresponding restriction sites of the pGEM3Z plasmid and reading frames are verified by

sequencing.

5. HIV-I env sequence corresponding to amino acids from 535 to 582 of the gp41 is amplified using 10 cycles of PCR from the pBH10 plasmid as described in Step 2. The primers for amplification are CCC tca GAG CTG TTG ATC CTT TAG G (nucleotides from 7337 to 7361 of the pBH10 sequence) and GCG TCt gaG ACG CTG ACG GTA CAG GCC (nucleotides from 7176 to 7202 of the pBH10 sequence). The PCR product is digested by XbaI restriction endonuclease followed by purification, cloning into the XbaI site of the pGEM3Z and sequencing to verify the reading frame.

6. The final cloned products are combined into the single construction and recloned as BamHI-SalI fragment into the eukaryotic expression vector pTK1 between BglII and XhoI sites downstream of the tk promoter and upstream of the SV40 polyadenylation signal followed by expression of the recombinant protein in transfected human lymphocytes.

While various embodiments and modifications of the invention have been described in the description, further variations will be apparent to those skilled in the art. Such modifications are included within

the scope of the present invention as defined by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Atkin, Andrew

(ii) TITLE OF INVENTION: RECOMBINANT VACCINE

(iii) NUMBER OF SEQUENCES: 6

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(E) COUNTRY: United States of America

(F) ZIP CODE: 03063

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette, 3.5-inch, 1.44 Mb storage

(B) COMPUTER: IBM PS/2 Model 30

(C) OPERATING SYSTEM: MS-DOS Version 3.30

(D) SOFTWARE: PFS: Professional Write

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT/US94/_____

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(viii) ATTORNEY/AGENT INFORMATION:

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(C) REFERENCE/DOCKET NUMBER: None

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
A A A T A C C t c T a G A G T G G G
          5         10        15
A G C C
  20
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

T C G G A T c C T C C C C A G A C G
5 10 15
C C G A G G A T G
20 25

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

C C C A C A G t C g a C T G T C T C
5 10 15

A G G C T T T
20 25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

G C T A C T A C A t C t A G A G C G
5 10 15

A G G C C G G G
20 25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

C C C t c a G A G C T G T T G A T C
5 10 15

C T T T A G G
20 25

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

G C G T C t g a G A C G C T G A C G
5 10 15

G T A C A G G C C
20 25

Claims

1. A method for an induction of immunity against target amino acid sequence within recombinant protein employing immune response against said target amino acid sequence, which target sequence substantially interacts with amino acid sequence of MHC product, wherein said interaction is achieved independently either [a] by direct fusion of the said target amino acid sequence with/within amino acid sequence of MHC product or [b] by fusion of said target sequence with an amino acid sequence binding noncovalently to MHC product or [c] by fusion with an amino acid sequence binding noncovalently to a cell membrane protein within the membrane cluster with MHC product or [d] by fusion with amino acid sequence of a cell membrane protein within the membrane cluster with MHC product.

2. A method according to Claim 1, where said recombinant protein is independently selected from group of recombinant proteins containing the target amino acid sequence within polypeptide and including either [a] hydrophobic transmembrane sequence(s) and

amino acid sequence(s) of MHC product providing interaction with T-lymphocyte MHC product receptor(s) or [b] amino acid sequence(s) binding to MHC product or [c] amino acid sequence(s) binding to a cell membrane protein(s) associating in a membrane cluster with MHC product(s) or [d] transmembrane sequence(s) and amino acid sequence(s) of cell membrane protein associating in membrane cluster with MHC product.

3. A method according to Claim 2, where said recombinant protein is produced by the expression of synthetic gene containing the coding nucleotide sequence of [a] the leader peptide sequence independently selected from the group of membrane proteins and/or secreted polypeptides, [b] target amino acid sequence, [c] MHC Class I sequence selected independently from the group of MHC Class I amino acid sequences of corresponding host and [d] transmembrane peptide sequence selected from the group of transmembrane peptide sequences of membrane proteins.

4. A method according to Claim 2, where said recombinant protein is produced by the expression of synthetic gene containing the coding nucleotide

sequence of [a] the leader peptide sequence independently selected from the group of membrane proteins and/or secreted polypeptides, [b] target amino acid sequence, [c] MHC Class II either alpha or beta chain sequence selected independently from the group of MHC Class II amino acid sequences of corresponding host and [d] transmembrane peptide sequence selected from the group of transmembrane peptide sequences of membrane proteins.

5. A method according to Claim 2, where said recombinant protein is produced by the expression of synthetic gene containing the coding nucleotide sequence of [a] the leader peptide sequence independently selected from the group of membrane proteins and/or secreted polypeptides, [b] target amino acid sequence, [c] beta-2 microglobulin sequence of immunized host and [d] transmembrane peptide sequence selected from the group of transmembrane peptide sequences of membrane proteins.

6. A method according to Claim 2, where said recombinant protein is produced by the expression of synthetic gene containing the coding nucleotide sequence of [a] the leader peptide sequence

independently selected from the group of consisting of membrane proteins and secreted polypeptides, [b] target amino acid sequence, [c] either MHC Class I or MHC Class II sequence of immunized host and [d] amino acid sequence selected from the group of the amino acid sequences of interleukines, lymphokines, cytokines, thymopoietins, thymosins, polypeptide hormons and other polypeptide ligands interacting with corresponding receptors on the cell membrane of corresponding immunized host.

7. A method according to Claim 1, where said recombinant protein is expressed using recombinant expression vectors for cell infection or transfection independently selected from the group of vaccinia virus vectors, recombinant viral vectors, bacterial plasmid vectors, bacterial phage vectors, yeast vectors and baculovirus vectors.

8. A method according to Claim 7, where said recombinant protein is expressed in cells of immunized host which are either infected by recombinant expression viral vector or transfected by recombinant DNA containing the nucleotide coding

sequence of said recombinant protein in vitro and said cells expressing said recombinant protein are used for immunization of the said host.

9. A method according to Claim 7, where said recombinant protein is expressed in cells of immunized host which are either infected by recombinant expression viral vector or transfected by recombinant DNA containing the nucleotide coding sequence of said recombinant protein in vivo.

10. A method according to Claim 9, where said recombinant protein is expressed in cells of immunized host transfected in vivo using recombinant DNA coding the amino acid sequence of said recombinant protein and said DNA is either bound to or incapsulated into vesicles selected from the group of liposomes, membrane of erythrocytes and erythrocyte ghosts, membrane of nucleated cells, vesicles and fragments of cell membrane, membrane of coated viruses and viral capsids and said vesicles present on the surface either [a] antibodies against cell membrane proteins or [b] peptides selected from the group of the amino acid sequences of interleukines, lymphokines, cytokines, thymopoietins,

thymosins, polypeptide hormons and other ligands interacting with corresponding receptors on the cell membrane of corresponding host and/or [c] either fusogenic protein or polypeptide or peptide derived from amino acid sequence of viral proteins selected from the group of fusogenic proteins of retroviruses, orthoviruses, paramyxoviruses, myxoviruses and coronaviruses containing the fusion sequence.

11. A method according to Claim 7, where said recombinant protein is produced in an expression system selected from the group of eukaryotic, prokaryotic, yeast and insect expression systems and either bound to surface of or incorporated into the natural and/or artificial membrane of vesicles selected from the group of liposomes, membrane of erythrocytes and erythrocyte ghosts, membrane of nucleated cells, vesicles and fragments of cell membrane, membrane of coated viruses and surface of viral capsids.

12. A method according to Claim 11, where said recombinant protein is either incorporated into membrane or bound to surface of liposomes, erythrocyte ghosts and membrane vesicles either

carrying on surface or encapsulating mediators of immune response selected either independently or in combination from the group of interleukines, lymphokines, cytokines, thymopoietins, thymosins, popypeptide hormons, biologically active peptide fragments of said mediators and other peptides with biological activity of said mediators.

AMENDED CLAIMS

[received by the International Bureau on 21 September 1994 (21.09.94); original claims 1-12 cancelled; new claims 13-25 added (7 pages)

(Original Claims 1-12 are cancelled.)

13. (new) A recombinant vaccine for an induction of immunity against a target amino acid sequence within a recombinant protein, wherein said target amino acid sequence is a naturally occurring or synthetic non-MHC coding amino acid sequence and said recombinant protein is constructed using a fusion of the said target amino acid sequence with an amino acid sequence of a carrier amino acid sequence, said target amino acid sequence being fused with a carrier sequence and displaying properties of an allospecific antigenic determinant, said carrier amino acid sequence being an amino acid sequence derived from a sequence selected from the group consisting of: (a) major histocompatibility antigens Class I; (b) major histocompatibility antigens Class II; (c) an amino acid sequence binding noncovalently to said major histocompatibility antigens Class I or Class II; (d) an amino acid sequence of membrane protein within a membrane cluster with said major histocompatibility antigen Class I or Class II; (e) an amino acid sequence binding noncovalently to a membrane protein within the membrane cluster with

said major histocompatibility antigen Class I or Class II; and (f) a combination thereof, said recombinant membrane protein being exposed to an immune system of an immunized host in a membrane-bound form and employing the immunological mechanisms of the allospecific immune response.

14. (new) The recombinant vaccine according to Claim 13, wherein said recombinant protein contains the target amino acid sequence within a carrier polypeptide and includes a member selected from the group consisting of a hydrophobic transmembrane sequence and amino acid sequence of MHC product, an amino acid sequence binding to MHC product, an amino acid sequence binding to a cell membrane protein associating in a membrane cluster with MHC product, a transmembrane sequence and amino acid sequence of cell membrane protein associating in membrane cluster with MHC product, and a combination thereof.

15. (new) The recombinant vaccine according to Claim 14, wherein said recombinant protein is produced by the expression of a synthetic gene constructed from a coding nucleotide sequence of a leader peptide sequence independent-ly selected sequences of membrane proteins, secreted poly-

peptides and a combination thereof, the target amino acid sequence, a MHC Class I sequence selected independently from the group of MHC Class I amino acid sequences of the corresponding host and a transmembrane peptide sequence selected from the group of transmembrane peptide sequences of membrane proteins.

16. (new) The recombinant vaccine according to Claim 15, wherein said recombinant protein is constructed from an N-terminal amino acid sequence of the MHC Class I product starting from amino acid position -24 corresponding to the start of the leader peptide and having from 71 to 85 amino acids, target amino acid sequence having 80 to 140 amino acids and amino acid sequence of the MHC class I product from position between 165 and 185 to the stop codon of the nucleotide coding sequence.

17. (new) The recombinant vaccine according to Claim 14, wherein said recombinant protein is produced by the expression of a synthetic gene constructed from a coding nucleotide sequence of a leader peptide sequence independently selected from the group of sequences of membrane proteins, secreted polypeptides and a combination thereof, the target amino acid sequence, a MHC Class II sequence selected

independently from the group of alpha- and beta-polypeptides of MHC Class II amino acid sequences of a corresponding host and a transmembrane peptide sequence selected from the group of transmembrane peptide sequences of membrane proteins.

18. (new) The recombinant vaccine according to Claim 14, wherein said recombinant protein is produced by the expression of a synthetic gene constructed from a coding nucleotide sequence of a leader peptide sequence independently selected from the group of sequences of membrane proteins, secreted polypeptides and a combination thereof, the target amino acid sequence, the beta-2-microglobulin sequence of a corresponding host and a transmembrane peptide sequence selected from the group of transmembrane peptide sequences of membrane proteins.

19. (new) The recombinant vaccine according to Claim 14, wherein said recombinant protein is produced by the expression of synthetic gene constructed from a coding nucleotide sequence of a leader peptide sequence independently selected from the group of sequences of membrane proteins, secreted polypeptides and a combination thereof, the target amino acid sequence, a MHC sequence of a correspond-

ing host and amino acid sequence selected from the group of the amino acid sequences of interleukines, lymphokines, cytokines, thymopoietins, thymosins, polypeptide hormones and a combination thereof, interacting with corresponding receptors on the cell membrane of a corresponding host.

20. (new) The recombinant vaccine according to Claim 13, wherein said recombinant protein is expressed using recombinant expression vectors for the cell infection or transfection independently selected from the group of recombinant vectors consisting of vaccinia virus vectors, recombinant retroviral vectors, bacterial plasmid vectors, bacterial phage vectors, yeast vectors and baculovirus vectors.

21. (new) The recombinant vaccine according to Claim 20, wherein said recombinant protein is expressed in cells which are either infected by a recombinant expression viral vector or transfected by a recombinant DNA carrying the nucleotide coding sequence of the said recombinant protein in vitro, said cells expressing said recombinant protein being useful for immunizing the donor of said cells.

22. (new) The recombinant vaccine according to

Claim 20, wherein said recombinant protein is expressed in cells which are either infected in vivo by a recombinant expression viral vector or trans-fected in vivo by a recombinant DNA carrying the nucleotide coding sequence of said recombinant protein.

23. (new) The recombinant vaccine according to Claim 22, where said recombinant protein is expressed in cells transfected in vivo using a recombinant DNA coding the amino acid sequence of said recombinant protein and said DNA is either bound to, or encapsulated into, vesicles selected from liposomes, a membrane of erythrocytes and erythrocyte ghosts, a membrane of nucleated cells, vesicles and fragments of cell membrane, a membrane of coated viruses and viral capsids and said vesicles present on their surface either targeting antibodies against cell membrane proteins or peptides selected from a group of amino acid sequences consisting of interleukines, lymphokines, cytokines, thymopoietins, thymosins, polypeptide hormones and a combination thereof, interacting with corresponding receptors on the cell membrane of corresponding host and either fusogenic protein or polypeptide or peptide derived from amino acid sequence of viral proteins selected from the group of

fusogenic proteins consisting of retroviruses, orthoviruses, paramyxoviruses, myxoviruses, coronaviruses and a combination thereof, with the fusion sequence.

24. (new) The recombinant vaccine according to Claim 20, wherein said recombinant protein is produced in an expression system selected from eukaryotic, prokaryotic, yeast and insect expression systems and either bound to surface of, or incorporated into, a natural or artificial membrane of vesicles selected from the group consisting of liposomes, a membrane of erythrocytes and erythrocyte ghosts, a membrane of nucleated cells, vesicles and fragments of cell membrane, a membrane of coated viruses and a surface of viral capsids and a combination thereof.

25. (new) The recombinant vaccine according to Claim 24, wherein said recombinant protein is either incorporated into a membrane or bound to surface of liposomes, erythrocyte ghosts and membrane vesicles either carrying on a surface or encapsulating the mediators of immune response selected from the group consisting of interleukines, lymphokines, cytokines, thymopoietins, thymosins, polypeptide hormones, biologically active peptide fragments of said mediators, and a combination thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04653

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : IPC(5): A61K 37/02, 39/00; C12N 15/00, 15/09

US CL : 424/88, 89, 92; 435/69.3, 69.7; 514/2.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 89, 92; 435/69.3, 69.7; 514/2, 530/395, 402, 403.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

AUTOMATED PATENT SYSTEM, DIALOG FILES 5, 155, 351, 399: KEY WORDS: MAJOR HISTOCOMPAT?, VACCIN?, TRANSMEMBRAN?, ANCHOR?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,400,376 (SANDERSON ET AL.) 23 AUGUST 1983, ABSTRACT.	1-12
Y	US, A, 4,478,823 (SANDERSON ET AL.) 23 OCTOBER 1984, ABSTRACT.	1-12
Y	US, A, 4,861,707 (IVANOFF ET AL.) 29 AUGUST 1989, ABSTRACT.	1-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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